Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Phytochemical Screening And Antioxidant Activity Of Euryale Ferox Leaf Extract: An Untapped Botanical Resource

Varsha Chaudhary[1], Dhirendra Vishwakarma[1], Roohi Kesharwani[1], Paras Gupta[2], Vineet Srivastava[1], Priyanka Yadav[1], Shahnawaz Sameem[1] *

[1]Faculty of Pharmacy, United University, Rawatpur, Prayagraj, Uttar Pradesh, India, 211012
[2]United Institute of Pharmacy, Naini, Prayagraj, Uttar Pradesh, India, 211010
***Corresponding Author-**
Dr. Shahnawaz Sameem
Email Id- shahnawaz@uniteduniversity.edu.in, ssameem.dr@gmail.com, ORCID ID- 0000-0003-1670-4830

Cite this paper as: Varsha Chaudhary, Dhirendra Vishwakarma, Roohi Kesharwani, Paras Gupta, Vineet Srivastava, Priyanka Yadav, Shahnawaz Sameem (2025) Phytochemical Screening And Antioxidant Activity Of Euryale Ferox Leaf Extract: An Untapped Botanical Resource. *Journal of Neonatal Surgery*, 14 (32s), 5751-5756.

ABSTRACT

Oxidative stress caused by reactive oxygen species (ROS) is a major contributor to the pathogenesis of several chronic diseases. Euryale ferox, traditionally recognized for its nutritional and medicinal properties, has been extensively studied for its seeds, while its leaves remain largely unexplored. This study investigates the antioxidant potential of E. ferox leaf extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Leaves were collected, shade-dried, and extracted with 70% ethanol. Antioxidant activity was evaluated by measuring DPPH radical scavenging, with ascorbic acid as the reference standard. The extract exhibited a concentration-dependent free radical scavenging effect. The IC_{50} value of the E. ferox leaf extract was found to be 71.18 ± 0.0691 µg/mL compared to 19.21±0.051 µg/mL, for ascorbic acid, indicating moderate to strong antioxidant activity. These results suggest that E. ferox leaves contain significant levels of polyphenolic and flavonoid compounds responsible for their antioxidant activity, supporting their potential use as a natural antioxidant in pharmaceutical and nutraceutical formulations.

Keywords: Euryale ferox, DPPH, antioxidant, leaf extract, polyphenols, radical scavenging

1. INTRODUCTION

Oxidative stress results from an overabundance of reactive oxygen species (ROS) and inadequate antioxidant defence and significantly contributes to the development of various chronic and degenerative disorders (1). These encompass cardiovascular diseases, diabetes mellitus, cancer, rheumatoid arthritis, and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (2,3). Reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, are generated during standard cellular metabolic activities. However, when their accumulation exceeds physiological levels, it leads to lipid peroxidation, DNA damage, protein oxidation, and ultimately, cell death. Therefore, the identification and utilization of compounds that can effectively scavenge free radicals are of significant interest in preventive medicine and therapeutic intervention (4,5).

In recent years, plant-based antioxidants have garnered widespread attention due to their effectiveness, minimal side effects, and environmentally friendly nature. Phytochemicals such as flavonoids, polyphenols, alkaloids, and tannins have demonstrated significant antioxidant activity by virtue of their hydrogen-donating and electron-transfer capabilities (6,7). As part of ongoing efforts to explore novel sources of natural antioxidants, traditionally used medicinal and edible plants are being extensively investigated for their phytochemical and pharmacological potential (8,9).

Euryale ferox, known as fox nut, gorgon nut, or makhana, is an aquatic plant within the Nymphaeaceae family. It is widely cultivated in the wetlands of South and Southeast Asia, particularly in the Indian subcontinent and China (10,11). The seeds of E. ferox have been consumed for centuries as a nutritious food and are an integral component of Ayurvedic and Traditional Chinese Medicine systems. Traditionally, they have been used to manage kidney disorders, reproductive issues, and inflammatory conditions. Nutritional analysis reveals that E. ferox seeds are rich in protein, starch, dietary fiber, essential amino acids, and micronutrients such as magnesium and potassium (4). More importantly, recent studies have identified a range of bioactive compounds in E. ferox, including gallic acid, catechin, chlorogenic acid, and ferulic acid, which are known for their antioxidant properties (12,13).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging test is among the most reliable and extensively utilized in vitro methods for evaluating antioxidant efficacy. This method assesses a compound's or extract's ability to contribute

hydrogen atoms or electrons, which neutralizes DPPH free radicals (14,15). This process leads to a measurable decrease in absorbance, allowing for quantitative analysis. The simplicity, speed, and reproducibility of this assay make it a valuable tool for the preliminary screening of antioxidant capacity in plant extracts (16).

Given the traditional use of Euryale ferox and its phytochemical richness, it is imperative to scientifically validate its antioxidant potential using standardized in vitro assays. This study is meant to assess the scavenging of free radicals activity of E. ferox seed extract through the DPPH assay, to substantiate its use as a natural antioxidant. The findings may support the development of E. ferox-based formulations for use in functional foods, nutraceuticals, and complementary therapeutic systems (17,18)

2. Materials and Methods

2.1 Plant Material Collection

Fresh *Euryale ferox* leaves were collected from Madhubani, Biha

2.2 Preparation of Extract

The leaves were shade-dried and then ground into a coarse powder. This powdered material was extracted using 70% ethanol in a Soxhlet apparatus for 8 hours. After extraction, the mixture was filtered and concentrated. The dried extract was preserved at 4°C until it was analyzed.it was analyzed (19,20).

2.3. Preliminary Phytochemical Investigation

Phytochemicals are naturally occurring bioactive compounds that contribute to the therapeutic effects of medicinal plants. The current study aimed to conduct a **preliminary phytochemical screening** of *Euryale ferox* Salisb. leaf extract prepared using **70% ethanol** to identify major classes of secondary metabolites that may contribute to its biological activity (21).

Alkaloids: Detected using Mayer's and Dragendorff's reagents.

Flavonoids: Identified via the alkaline reagent and Shinoda tests.

Tannins and Phenols: Tested using 5% ferric chloride solution.

Saponins: Evaluated using the froth formation test.

Glycosides: Determined by the Keller–Kiliani test.

Steroids and Terpenoids: Identified using Liebermann–Burchard and

Salkowski tests.

Carbohydrates: Confirmed by Molisch's test.

Proteins: Assessed using Biuret's test.

Observations were recorded based on colour changes, precipitate formation, and frothing behaviour.

2.4 Chemicals and Reagents

All chemicals used in this study were of analytical grade. DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from SRL Chemicals (Cat. No. SR-29128), and methanol was procured from SD Fine-Chem Limited (Cat. No. 10930lC250). Ascorbic acid, used as the reference antioxidant, was also purchased from SRL (Cat. No. 23006).

2.5 Sample Preparation

The leaves of *Euryale ferox* were collected, shade-dried, and pulverized into a fine powder. The powdered material was subjected to extraction using 70% ethanol. The resulting extract was concentrated and stored at 4°C until further use. Different concentrations of the extract were prepared in ethanol for the assay.

2.6 DPPH Radical Scavenging Assay

The antioxidant capacity of the leaf extract was evaluated using the DPPH free radical scavenging method. The assay was conducted in a 96-well microplate. To each well, 10 µL of the test extract or standard (ascorbic acid) was added to 200 µL of freshly prepared 0.1 mM DPPH solution in methanol. All samples and standards were analyzed in quadruplicate to ensure reliability. Additionally, blank wells were prepared using 200 µL of methanol and 10 µL of the test solution, but without DPPH (22,23).

Wells containing only DPPH and methanol were considered the negative control. Another control set was included, where 20 µL of deionized water replaced the test solution. The plate was kept in the dark at room temperature for 30 minutes to allow the reaction to proceed without light interference. After incubation, absorbance was measured at 517 nm using a microplate reader (iMark, Bio-Rad) (24,25).

2.7 Data Analysis

The free radical scavenging activity was expressed as the percentage of inhibition compared to the control using the following formula:

Calculations

% RSA = ((Abs Control- Abs Sample)/Abs Control) ×100

RSA = Radical Scavenging Activity

Abs Control = Absorbance of control

Abs Sample = Absorbance of sample

The IC_{50} value, which represents the concentration required to inhibit 50% of the DPPH radicals, was calculated using GraphPad Prism version 5. A graph was plotted with concentration on the X-axis and percent inhibition on the Y-axis to estimate the IC_{50} values.

3. Results

3.1. Preliminary Phytochemical Screening

The phytochemical analysis of *Euryale ferox* leaf extract revealed the presence of various bioactive constituents. The results of standard qualitative chemical tests are summarized in Table 1.

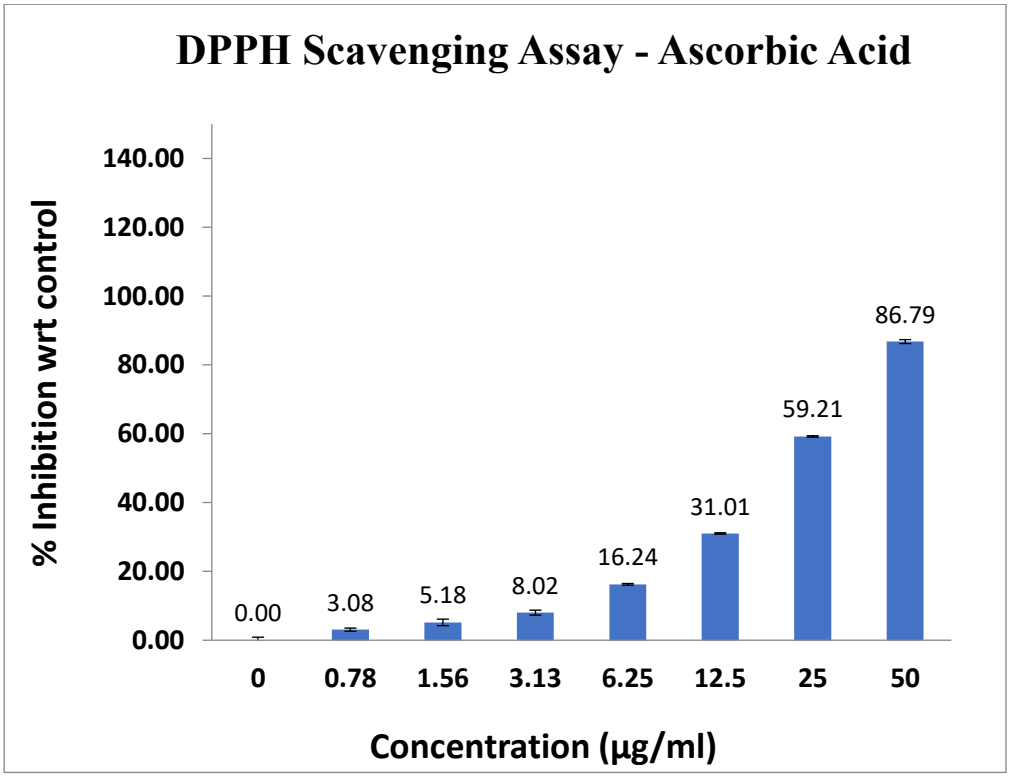
Table 1: Preliminary Phytochemical Screening of *Euryale ferox* Leaf Extract

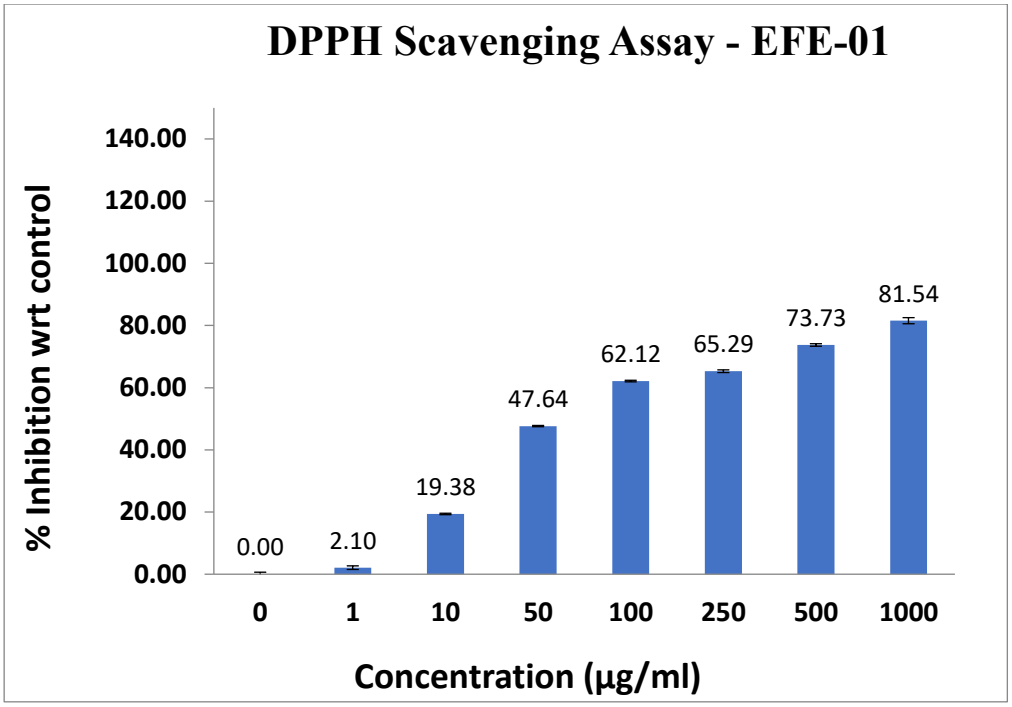
3.2. DPPH Radical Scavenging Activity

The *Euryale ferox* leaf extract demonstrated a concentration-dependent scavenging of DPPH radicals. As the extract concentration increased from 10 µg/mL to 100 µg/mL, the percentage inhibition of DPPH increased significantly.

The IC_{50} (half-maximal inhibitory concentration) of the *E. ferox* leaf extract was calculated to be **71.18 ± 0.0691 µg/mL** compared to **19.21±0.051 µg/mL**for ascorbic acid.

Figure 1 Graph showing % inhibition of DPPH radicals vs. concentration of *Euryale ferox* extract and ascorbic acid.





4. Discussion

The findings of this study provide evidence of the antioxidant potential of *Euryale ferox* leaf extract, as demonstrated by its free radical scavenging activity in the DPPH assay. The extract showed a strong dose-dependent inhibition of DPPH radicals, with an IC_{50} value of **71.18 ± 0.0691 µg/mL**, indicating moderate to strong antioxidant activity when compared with the standard antioxidant, ascorbic acid (18).

The initial phytochemical analysis indicated the presence of flavonoids, phenolics, alkaloids, and glycosides in the extract. These types of chemicals are recognized for their capacity to transfer hydrogen or electrons which is the primary mechanism in neutralizing free radicals like DPPH. Phenolic compounds, in particular, are effective in stabilizing free radicals due to the resonance-stabilized structure of their aromatic rings. The presence of saponins and terpenoids may also enhance the antioxidant effect through synergistic action with polyphenols (26).

The comparatively higher IC_{50} value of the leaf extract than that of ascorbic acid may be due to the presence of a mixture of antioxidant compounds, which individually may be less potent but collectively contribute to the extract's efficacy. Furthermore, crude extracts often show reduced potency due to the presence of non-active constituents or antagonistic interactions (27).

These results suggest that *Euryale ferox* leaves can serve as a natural and accessible source of antioxidants, which could be beneficial in managing oxidative stress-related conditions, especially in neonatal health where oxidative injury can contribute to complications such as bronchopulmonary dysplasia, retinopathy of prematurity, and periventricular leukomalacia

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